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A Study of Lipids and Water of Liver and Muscle in *Fundulus heteroclitus* (Linnaeus) and *Stenotomus versicolor* (Mitchill)[1]

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Introduction

The present study provides biochemical data for two common species from the temperate zone, the scup or porgy, *Stenotomus versicolor* (Mitchill), and the killifish or mummichog, *Fundulus heteroclitus* (Linnaeus). Although the role of tissue water and lipid composition has been considered (Gueylard, 1924, and Bloor, 1943), there does not appear to be any recent study of the subject in relation to fish tissues. The role of tissue lipids and their relationship to water content is appraised, and from a comparative viewpoint the data from a series of studies on arctic fishes have been used in discussion and evaluation in the present work.

Material and Methods

The facilities of the Marine Biological Laboratory at Woods Hole were used to obtain freshly-caught scup and killifish. Specimens were transferred to aquaria with fresh circulating sea water. Only active, vigorous specimens were selected for study. Each fish was spinalectomized at the base of the skull, and liver and muscle were excised in a matter of minutes. The pieces of tissue were removed from the same loci in every instance. Myotomic muscle of the scup was taken from a lateral area immediately posterior to the pectoral fin, and wedges of liver were taken from the same ventro-caudad marginal area each time. In the killifish, because of its small size, a slender strip of the left fillet was used for water analysis and a similar piece from the right fillet for fat extraction.

Tissue for water extraction was weighed on tarred pieces of aluminum foil and dried to constant weight. One hundred degrees centigrade for 22 hours, followed by two more weighings within six hours, was found to be sufficient to obtain a constant weight. From each scup, one to three pieces were weighed and dried. When more than one piece of tissue was taken from a given animal, the values were averaged. The dry weight method, because of its common usage, was selected in preference to others.

The methods used in the lipid analyses are identical with those cited in two earlier reports (Wilber & Musacchia, 1951, and Musacchia, Sullivan & Wilber, 1957).

Results

There is a notable similarity in the water content of the liver and the muscle from scup and killifish (Table 1). The water is in highest concentration in the muscle tissue of each species, and conversely the lipids are more concentrated in the liver. Although the cholesterol and total fatty acid concentration are highest in the liver of killifish, the ratio, cholesterol/total fatty acid, is almost identical for liver from both species: killifish, 0.080, and scup, 0.088. Table 1 shows that killifish liver has about twice as much total lipids or individual lipid components as scup liver. On the other hand, the phospholipid content of muscle from both species was found to be quite similar, in percent. of fresh tissue: killifish, 0.904, and scup, 0.951.

The summarized results (Table 1) suffice to illustrate in general differences in the two species, the killifish a euryhaline fish and scup a marine fish.

Discussion

Evaluation of the concentration of water in the tissues of fishes is essential to a better understanding of their biochemistry. Vinogradov (1953) devotes considerable attention to this

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Table 1. Lipid Values for Liver and Muscle from Killifish and Scup
The values are percentages of fresh tissue; means and mean deviations are given.

Species	Cholesterol	Phospholipid	Total fatty acid	Total lipids	C/FA[1]	C/P[2]	H_2O
Stenotomus versicolor							
liver (18)[3]	0.386	3.345(19)	4.64	5.017(17)	0.088(17)	0.126(17)	73.372
	0.084	0.060	0.12	1.330	0.023	0.032	1.408
muscle (20)	0.091	0.951	2.03	2.121	0.051	0.096	79.299
	0.013	0.086	0.56	0.567	0.015	0.016	1.597
Fundulus heteroclitus							
liver (15)	0.799	6.972	10.14	10.909	0.080	0.107	71.458
	0.218	1.116	1.26	1.447	0.021	0.035	3.477
muscle (15)	0.133	0.904	1.60	1.732	0.085	0.170	78.998
	0.023	0.194	0.25	0.351	0.008	0.058	1.846

[1] C/FA = cholesterol/total fatty acid.
[2] C/P = cholesterol/phospholipid.
[3] Unless otherwise noted, the number of specimens analyzed is given in the first column.

subject. He lists values for the water content of muscle from 25 species of the Family Sparidae and, in general, the water content is about 75 percent. or less. The values range from 73.55 to 88.63 (in percent. of living matter) and, more specifically, for muscle of *Stenotomus argyrops,* 74.94 percent. and *Stenotomus chrysops* (= *S. versicolor*), 76.61 percent. In the present investigation, the mean value for water concentration in muscle of *Stenotomus versicolor* is 79.299 percent. This value is comparable with those of other members of the Family Sparidae; it is, however, slightly higher than the values reported by Vinogradov for the genus *Stenotomus.* Vinogradov expresses the view that in fish tissue the amount of water varies inversely with the amount of fat. The data obtained for scup and killifish tend to support this opinion. It is apparent (Table 1) that water is in greater concentration in muscle where total fatty acids are lower and, conversely, in liver total fatty acids are higher and water is in less concentration.

Some of the differences in the two species studied warrant discussion. For example, the liver tissue of killifish has almost double the cholesterol value of the liver of scup. The cholesterol content of muscle in killifish is in greater concentration in both absolute quantity and relationship to total fatty acid and phospholipid contents. According to some investigators, tissue cholesterol and tissue water are intimately associated. Mayer & Schaeffer (1914 a and b) reported a direct relationship between the ratio of cholesterol/total fatty acids and the water of imbibition. Cholesterol mobilization may be considered responsible for maintenance of normal water content of tissues in the euryhaline stickleback, *Gasterosteus pungitius,* (Gueylard, 1924). In his 1943 monograph, Bloor gives further discussion of the subject. It is evident from the data presented in Table 1 that there is a relative constancy in the water content of comparable tissues from scup and killifish, and at the same time there are gross differences in cholesterol content in the same tissues. These findings tend to weaken the hypothesis that the role of tissue cholesterol is to influence water concentration.

The relation of lipid phosphorus to cholesterol, *i.e.*, cholesterol/lipid phosphorus, has also been associated with the capacity of tissue to hold water (Mayer & Schaeffer, 1913, 1914 a and b). Again, from the evidence obtained, no correlation is apparent between the water content and the ratio, cholesterol/phospholipid. The concentration of phospholipid in muscle is similar in both species, but the ratio, cholesterol/phospholipid, is almost twice as great in *F. heteroclitus.* The gross hiatus in ratio values does not have a counterpart in the values for water content. With regard to the lipid ratios and the tissue water content values, these two species fail to provide evidence to support some of the contentions of Mayer & Schaeffer (*loc. cit.*).

Mayer & Schaeffer postulated that the *coefficient lipocytique,* cholesterol/total fatty acids, is constant in tissues from animals of diverse species. The values derived from the livers of scup and killifish, 0.088 (0.023) and 0.080 (0.021) respectively, are nearly the same, while those obtained for muscle are of the same order of magnitude, 0.051 (0.015) and 0.085 (0.008)

respectively. The data presented thus tend to confirm the reality of a lipocytic coefficient, and at least one other instance of supportive evidence can be found in earlier work where the muscle from *Boreogadus saida* (the arctic or polar tomcod) was reported as 0.088 ± 0.022 (Musacchia, Sullivan & Wilber, 1957).

Bloor (1943) shows that phospholipid values for muscle vary widely not only with the type of muscle but also with the animal. It is therefore of interest that the phospholipid values obtained for myotomic muscle of a variety of fishes possess some similarity: *Mallotus catervarius* 1.2, *Boreogadus saida* 1.7 (Musacchia, Sullivan & Wilber, 1957), *Myoxocephalus quadricornis* 1.85 (Musacchia & Clark, 1957), *Fundulus heteroclitus* 0.9, and *Stenotomus versicolor* 0.95 (all values expressed in percent. of fresh tissue). Some 30 years ago, muscle phospholipid values of the same order of magnitude were reported (in grams per hundred grams) for carp as 1.1 and dogfish as 1.1 (Javillier, *et al.*, 1928). These similarities are of value in further consideration of the role of phospholipid in muscle physiology.

Summary

1. Cholesterol, phospholipid, total fatty acid and water content of the liver and muscle of two common temperate zone fish, *Stenotomus versicolor* and *Fundulus heteroclitus*, have been determined, and the relationships of the various lipids to each other and to water content have been shown.

2. In both species, the muscle tissue has a higher water content than the liver, while the latter has the higher lipid concentration. An inverse relationship of tissue water and lipid concentrations is demonstrated.

3. Evidence is presented that tends to confirm the existence of a lipocytic coefficient, *i.e.*, that the cholesterol/total fatty acid ratio is constant in tissues from diverse species of fishes.

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